

Comparison of survival and development of *Lycaeides argyrognomon* (Bergsträsser) (Lepidoptera: Lycaenidae) reared on two different food plants, *Indigofera pseudo-tinctoria* and Chinese-grown *Indigofera* sp.

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Abstract The larvae of *Lycaeides argyrognomon* (Bergsträsser) feed only on *Indigofera pseudo-tinctoria* (JPN-I). A Chinese-grown *Indigofera* sp. (CHA-I) has been used in recent times for revegetation of roadside slopes. This study was carried out to confirm that *L. argyrognomon* larvae given CHA-I can grow well and to compare the survival and development of *L. argyrognomon* reared on the two different *Indigofera* plants. Newly hatched larvae were divided into two groups, one given JPN-I leaves and the other given CHA-I leaves and kept in an incubator at a constant temperature of 25°C with a photoperiod of 16L : 8D. The means of the developmental periods of males were shorter than those of females reared on JPN-I and CHA-I. There were no significant differences in the survival rate, the rate of abnormal emergence or the developmental periods between the group reared on JPN-I and that reared on CHA-I. The means of the pupal weight and the forewing length in individuals reared on CHA-I were larger than those of individuals reared on JPN-I. The possibility of using CHA-I as host plants for *L. argyrognomon* is discussed.

Key words adult forewing, host plant, developmental period, *Lycaeides argyrognomon*, the weight of pupa.

Introduction

Lycaeides argyrognomon (Bergsträsser) is a grassland lycaenid butterfly distributed in Japan, the Korean Peninsula, Northeastern China, Europe and North America (Shirozu, 2006). Since 1970s, the habitats of *L. argyrognomon* have rapidly decreased in Japan. Large populations of this butterfly were once found throughout the Nagano Prefecture, but it has now become extinct in the northern part of the Prefecture (Tashita *et al.*, 1999). Thus, this butterfly was designated Vulnerable (VU) by the Ministry of the Environment (2007) and Near Threatened (NT) by Nagano Prefecture (2004).

Many leguminous plants have been reported as host plants of *L. argyrognomon* larvae in Europe and North America (Takahashi, 2007). In contrast, in Japan larvae feed only on *Indigofera pseudo-tinctoria*, with a few exceptional reports that eggs and larvae were found on *Hedysarum vicioides* (Fukuda *et al.*, 1984). Kimura and Ono (1975) pointed out that *L. argyrognomon* might use *Indigofera decora* or *Indigofera kirilowii* as host plants, but there are no reports confirming these two *Indigofera* plants as hosts of *L. argyrognomon*.

Native *I. pseudo-tinctoria* of Japan (JPN-I) was applied to revegetational technology of roadside slopes through the 1980s. However, in 1990 and afterwards, seeds produced in China were imported for this purpose, resulting in Chinese-grown *Indigofera* sp. (CHA-I) growing along roadside slopes (Aiba and Natsume, 1995; Yoshida, 2008).

The scientific name of CHA-I has not been yet established (Uemura *et al.*, 2010). The mean height of CHA-I is 3 m or more, in contrast to JPN-I with a mean height of 0.5–0.9 m (Osada, 1981; Yoshida and Morimoto, 2005). There is no obvious morphological difference in leaf or flower between JPN-I and CHA-I. In such a situation, the possibility that *L. argyrognomon* larvae feed on CHA-I obviously exists.

The objectives of this study were to confirm that *L. argyrognomon* larvae given CHA-I can grow well and to compare the survival and development of *L. argyrognomon* reared on the two different *Indigofera* plants.

Materials and methods

Insects

Adult females of *L. argyrognomon* were collected at several spots along the Otagiri River in Komagane City, Nagano Prefecture, Japan, in June 2010. These females were put in a cylindrical cage (220 mm in diameter, 150 mm high) made of polyester cloth at room temperature with a photoperiod of 16L : 8D in the laboratory of the Faculty of Agriculture, Shinshu University, and fed sugared water as nectar. In this study, we used eggs laid on JPN-I by these females from June 6 to June 10, 2010.

Food plants

JPN-I (Fig. 1A) was collected at the campus of the Faculty

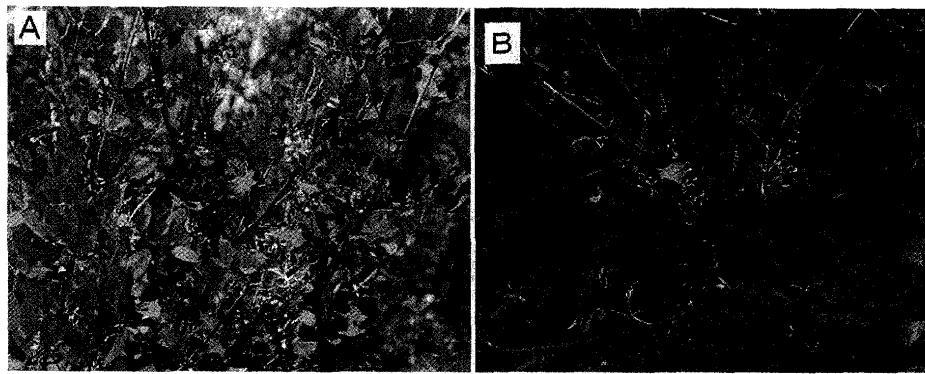


Fig.1 *Indigofera pseudo-tinctoria* (JPN-I) at the campus of the Faculty of Agriculture, Shinshu University (A) and Chinese-grown *Indigofera* sp. (CHA-I) at a roadside slope in Minowa Town, Nagano Prefecture (B).

Table 1. Survival rates of larval and pupal stages and the sex ratio of *Lycaeides argyrognomon* reared on different food plants.

| Food plant | Number of individuals | | | Survival rate (%) | | Sex ratio of adult $\delta/(\delta+\delta)$ |
|---|-----------------------|------|-------|-------------------|-------------|--|
| | First instar | Pupa | Adult | Larval stage | Pupal stage | |
| <i>Indigofera pseudo-tinctoria</i> (JPN-I) | 43 | 29 | 28 | 67.4 | 96.6 | 0.571 |
| <i>Indigofera</i> sp. (CHA-I) | 65 | 45 | 45 | 69.2 | 100.0 | 0.444 |

Table 2. Percentages of abnormally emerged adults of *Lycaeides argyrognomon* reared on different food plants.

| Food plant | δ | | δ | | % of abnormal emergence |
|---|------------------|--------------------|------------------|--------------------|-------------------------|
| | Normal emergence | Abnormal emergence | Normal emergence | Abnormal emergence | |
| <i>Indigofera pseudo-tinctoria</i> (JPN-I) | 15 | 1 | 11 | 1 | 7.14 |
| <i>Indigofera</i> sp. (CHA-I) | 17 | 3 | 24 | 1 | 8.89 |

of Agriculture, Shinshu University, and CHA-I (Fig. 1B) was collected from a roadside slope in Minowa Town, Nagano Prefecture, where CHA-I was planted artificially for slope revegetation.

Rearing methods

Newly hatched larvae were divided into two groups, one given JPN-I leaves and the other given CHA-I leaves. Forty-six larvae of the JPN-I group were reared in three Petri dishes (90 mm in diameter, 40 mm high) of 16, 15 and 15 larvae, and 65 larvae of the CHA-I group were reared in five Petri dishes of 9, 11, 15, 15 and 15 larvae. These Petri dishes were kept in an incubator at a constant temperature of 25°C with a photoperiod of 16L: 8D. Fresh leaves of food plants were given at intervals of two or three days.

After pupation, each pupa was placed in a new Petri dish of the same size with a filter paper. The mortality and development of the larvae and pupae were examined daily. The weight of each pupa was measured using an electronic

chemical balance (Chyo JL-180) within one day after pupation, and the length of each adult forewing was measured using vernier calipers after spreading the wings on a setting board.

Statistical analysis

For statistical analysis, the larval and pupal periods, the weight of pupae and the length of adult forewings were analyzed using two way repeated measures ANOVA (analysis of variance) and Scheffe's multiple comparisons considering the two factors of the food plants and sex. Survival rates, sex ratio and abnormal emergence rates were analyzed by the χ^2 test.

Results

Survival rates of larval and pupal stages Table 1 shows survival rates of the larval and pupal stages reared on the different food plants. The survival rate at the larval stage was 67.4% on JPN-I and 69.2% on CHA-I, which was not a significant difference (χ^2 test, $p=0.987$). The survival

Table 3. Means of larval and pupal developmental periods of *Lycaeides argyrognomon* reared on different food plants.

| Food plant | Sex | Larval period | | Pupal period | |
|--|-------|-------------------|----------------------|-------------------|----------------------|
| | | Number of samples | Mean \pm SD (days) | Number of samples | Mean \pm SD (days) |
| <i>Indigofera pseudo-tinctoria</i> (JPN-I) | ♂ | 16 | 17.00 \pm 0.72 | 16 | 7.50 \pm 0.88 |
| | ♀ | 12 | 18.33 \pm 0.90 | 10 | 8.20 \pm 0.63 |
| | Total | 28 | 17.57 \pm 1.06 | 26 | 7.77 \pm 0.87 |
| <i>Indigofera</i> sp. (CHA-I) | ♂ | 20 | 16.40 \pm 1.43 | 20 | 7.55 \pm 0.67 |
| | ♀ | 25 | 18.72 \pm 1.25 | 24 | 7.83 \pm 0.78 |
| | Total | 45 | 17.69 \pm 1.74 | 44 | 7.70 \pm 0.73 |
| Total | ♂ | 36 | 16.67 \pm 1.20 | 36 | 7.53 \pm 0.78 |
| | ♀ | 37 | 18.59 \pm 1.16 | 34 | 7.94 \pm 0.76 |

Table 4. Means of the weight of pupae and the length of adult forewings of *Lycaeides argyrognomon* reared on different food plants.

| Food plant | Sex | Pupal weight | | Length of adult forewing | |
|--|-------|-------------------|----------------------|--------------------------|----------------------|
| | | Number of samples | Mean \pm SD (days) | Number of samples | Mean \pm SD (days) |
| <i>Indigofera pseudo-tinctoria</i> (JPN-I) | ♂ | 16 | 51.70 \pm 3.80 | 16 | 13.01 \pm 0.44 |
| | ♀ | 12 | 53.33 \pm 3.91 | 12 | 13.06 \pm 0.55 |
| | Total | 28 | 52.40 \pm 3.86 | 28 | 13.03 \pm 0.48 |
| <i>Indigofera</i> sp. (CHA-I) | ♂ | 20 | 64.39 \pm 7.33 | 20 | 13.97 \pm 0.67 |
| | ♀ | 25 | 60.04 \pm 7.25 | 25 | 13.66 \pm 0.80 |
| | Total | 45 | 61.97 \pm 7.51 | 45 | 13.79 \pm 0.74 |
| Total | ♂ | 36 | 58.75 \pm 8.76 | 36 | 13.54 \pm 0.72 |
| | ♀ | 37 | 57.86 \pm 7.06 | 37 | 13.46 \pm 0.79 |

rate of the pupal stage was 96.6% in pupae reared on JPN-I and 100% in those reared on CHA-I, which was not a significant difference (χ^2 test, $p=0.824$).

Sex ratio

The sex ratio of adults was 0.571 ♂ in *L. argyrognomon* reared on JPN-I and 0.444 ♂ in those reared on CHA-I, which was not a significant difference (χ^2 test, $p=0.415$) (Table 1).

Rate of abnormal emergence adults

The percentages of abnormally emerged adults which did not extend their wings were 7.14% on JPN-I and 8.89% on CHA-I (Table 2). This was not a significant difference (χ^2 test, $p=0.862$).

Developmental periods of larval and pupal stages

Larvae were reared in several rearing groups. There were no significant differences in means of the larval and pupal periods among rearing groups between JPN-I (the two-way ANOVA: larval period, $p=0.352$; pupal period, $p=0.580$) and CHA-I (the two-way ANOVA: larval period, $p=0.958$; pupal period, $p=0.372$).

The developmental periods of the larvae and pupae reared on JPN-I and CHA-I are summarized in Table 3. Results of the two-way ANOVA on the two main factors of food plant and sex, and Sheffe's multiple comparison are shown in Table 5. Larval periods of males and females showed a significant difference in two-way ANOVA ($p<0.001$), but the kind of food plants did not affect the larval period in two-way ANOVA ($p=0.647$). As for the interaction on the larval period between the two factors, it was not statistically significant (two-way ANOVA, $p=0.084$) (Table 5).

Means of pupal periods for pupae reared on JPN-I and CHA-I were 7.77 and 7.70 days, respectively, and those of males and females were 7.53 and 7.94 days, respectively (Table 3). While pupal periods of males and females showed a significant difference (two-way ANOVA, $p=0.026$), the difference in food plant species did not affect the pupal period (two-way ANOVA, $p=0.481$). As for the interaction on the pupal period between the two factors, it was not statistically significant (two-way ANOVA, $p=0.279$) (Table 5).

Weight of pupae and length of adult forewings

No significant differences in the weight of pupae or the

Table 5. Two-way ANOVA table and Sheffe's multiple comparison on larval and pupal developmental periods, pupal weight and adult forewing length of *Lycaeides argyrognomon*.

| Variables measured | Source of variation | Two-way ANOVA | | Sheffe's multiple comparison | | |
|--------------------------|---------------------|---------------|---------|------------------------------|---|--------------|
| | | F | p-value | Means compared | Difference of means | p-value |
| Larval period | Sex | 50.587 | <0.001 | S* | $\delta \times \eta$ | 1.928 <0.001 |
| | Food plant | 0.212 | 0.647 | | <i>I. pseudo-tinctoria</i> (JPN-I) \times <i>I. sp.</i> (CHA-I) | 0.117 0.675 |
| | Interaction | 3.079 | 0.084 | | | |
| Pupal period | Sex | 5.206 | 0.026 | S | $\delta \times \eta$ | 0.413 0.026 |
| | Food plant | 0.502 | 0.481 | | <i>I. pseudo-tinctoria</i> (JPN-I) \times <i>I. sp.</i> (CHA-I) | -0.065 0.731 |
| | Interaction | 1.190 | 0.279 | | | |
| Pupal weight | Sex | 0.369 | 0.546 | | $\delta \times \eta$ | -0.885 0.546 |
| | Food plant | 42.433 | <0.001 | S | <i>I. pseudo-tinctoria</i> (JPN-I) \times <i>I. sp.</i> (CHA-I) | 9.575 <0.001 |
| | Interaction | 3.899 | 0.052 | | | |
| Length of adult forewing | Sex | 0.256 | 0.615 | | $\delta \times \eta$ | -0.078 0.615 |
| | Food plant | 24.004 | <0.001 | S | <i>I. pseudo-tinctoria</i> (JPN-I) \times <i>I. sp.</i> (CHA-I) | 0.762 <0.001 |
| | Interaction | 1.212 | 0.275 | | | |

*: S indicates significant difference at the level of 5 %.

length of adult forewings were found in the rearing groups on JPN-*I* (two-way ANOVA: weight, $p=0.090$; forewing, $p=0.098$) and CHA-*I* (two-way ANOVA: weight, $p=0.415$; forewing, $p=0.744$).

The weight of pupae and the length of adult forewings reared on JPN-*I* and CHA-*I* are summarized in Table 4. Results of the two-way ANOVA on the two main factors of food plant and sex, and Sheffe's multiple comparison are shown in Table 5. Mean weights of pupae reared on JPN-*I* and CHA-*I* showed a significant difference (two-way ANOVA, $p<0.001$), but the sex did not affect the pupal weights (two-way ANOVA, $p=0.546$). As for the interaction on the pupal weight between the two factors, it was not statistically significant (two-way ANOVA, $p=0.052$) (Table 5).

The means of forewing length of adults reared on JPN-*I* and CHA-*I* were 13.03 and 13.79 mm, respectively, and those of males and females were 13.54 and 13.46 days, respectively (Table 4). While forewing length of adults reared on JPN-*I* and CHA-*I* showed a significant difference (two-way ANOVA, $p<0.001$), the sex did not affect the forewing length (two-way ANOVA, $p=0.615$). As for the interaction on the forewing length between the two factors, it was not statistically significant (two-way ANOVA, $p=0.275$) (Table 5).

Discussion

Differences of developmental periods between males and females

Earlier emergence of male butterflies has been generally observed (e.g. Watanabe, 1978; Koda and Nakamura, 2010). In this study, the means of the developmental periods of males were shorter than those of females (Table 3). We also observed by a transect census of *L. argyrognomon* in Ina City that the peak occurrence of male butterflies was earlier than that of females (Fujiwara *et al.*, unpublished). This may be caused by the differences of developmental periods between males and females shown in the present study.

Food plants of *L. argyrognomon* larvae

Takahashi (2007) reported that *L. argyrognomon* larvae from the Korea Peninsula and the Maritime Province of Siberia ate *Vicia amurensis* but that they were unable to feed on *I. pseudo-tinctoria* (JPN-*I*); however, *L. argyrognomon* larvae in Japan are unable to feed on *V. amurensis*. In the case of *L. argyrognomon*, local populations may have adjusted to the differences in the constituents of the food plants, leading to hereditary specialization in these populations.

Yago (2007) reported a few host plants of *L. argyrognomon* in addition to JPN-*I*. Larvae were found to feed on *Astrogalus membranaceus* and *Glycine max*. Some adults were observed to lay eggs on *Hedysarum vicioides*. This indicates that *L. argyrognomon* larvae may develop on other plants besides JPN-*I*. In this study, we reared *L. argyrognomon* larvae on two *Indigofera* plants, JPN-*I* and CHA-*I*. The survival rate and development period were not influenced by the difference in food plants (Tables 1 and 3). The means of the pupal weight and the forewing length in individuals reared on CHA-*I* were larger than those of JPN-*I*. These results were the same for both females and males (Table 4).

According to allozyme analysis, the degree of hereditary sameness of JPN-*I* and CHA-*I* was 0.46–0.57, and therefore, these two food plants were judged to be different species belonging to the same genus, *Indigofera* (Abe *et al.*, 2004). It became clear from the results of the present study that CHA-*I* can be used as a food plant when rearing *L. argyrognomon* larvae.

The possibility of using CHA-*I* as a host plant

Larvae of *Eurema laeta* (Boisduval) were considered to feed on only *Chamaecrista nomame* in Japan for a long time (Shirozu, 2006). As *E. laeta* disappeared in many habitats with a corresponding decrease of *C. nomame*, the latter was designated Vulnerable (VU) by the Ministry of the Environment (2007). However, recently it was reported that the populations of *E. laeta* in Shizuoka, Aichi and Gifu Prefectures have increased because of host conversion from *C. nomame* to *Chamaecrista nictitans*, which is a naturalized plant (Ueyama, 2009).

It is known that *Zizina otis* (Fabricius), a threatened butterfly in Japan, increased in Osaka and Hyogo Prefectures by host conversion from *Lotus corniculatus* to *Trifolium repens* (Ishii *et al.*, 2008).

In recent times, CHA-*I* has been imported from China to use for slope revegetation in Japan. There is an obvious probability that *L. argyrognomon* may convert its host plant from JPN-*I* to CHA-*I*, as in the case of *E. laeta* and *Z. otis*. The following two points may be deciding factors in determining whether *L. argyrognomon* can use CHA-*I* as a host plant: 1) Does the adult butterfly lay eggs on CHA-*I*? 2) Does the adult butterfly move around its habitat and find CHA-*I*? Further research is necessary to clarify these two points and to investigate whether *L. argyrognomon* is found in the areas where CHA-*I* is growing.

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摘 要

在来コマツナギと中国産コマツナギで飼育されたミヤマシジミの生存と発育の比較 (江田慧子・中村寛志)

ミヤマシジミの食草は在来コマツナギであるが、近年中国産コマツナギが道路法面の緑化に使われるようになってきた。本研究では、ミヤマシジミ幼虫が中国産コマツナギを摂食して正常に成長するかどうかを確認し、その生存率と発育状態を在来コマツナギを食べた個体と比較した。孵化直後のミヤマシジミの幼虫を在来コマツナギ食43個体と中国産コマツナギ食65個体のグループに分け、25°C、16L: 8Dの恒温器で成虫まで飼育した。♂の発育期間は♀より約2日ほど早かった。生存率、羽化不全率、発育期間に関しては、在来コマツナギ食と中国産コマツナギ食では差がみられなかった。蛹体重と前翅長の平均値は、在来コマツナギ食より中国産コマツナギ食の方が大きかった。これらの結果から、ミヤマシジミが中国産コマツナギを食草にする可能性を考察した。

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